Supplementary Material for

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"The role of HSP70 on ENPP1 expression and insulin receptor activation"
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SUPPLEMENTARY METHODS

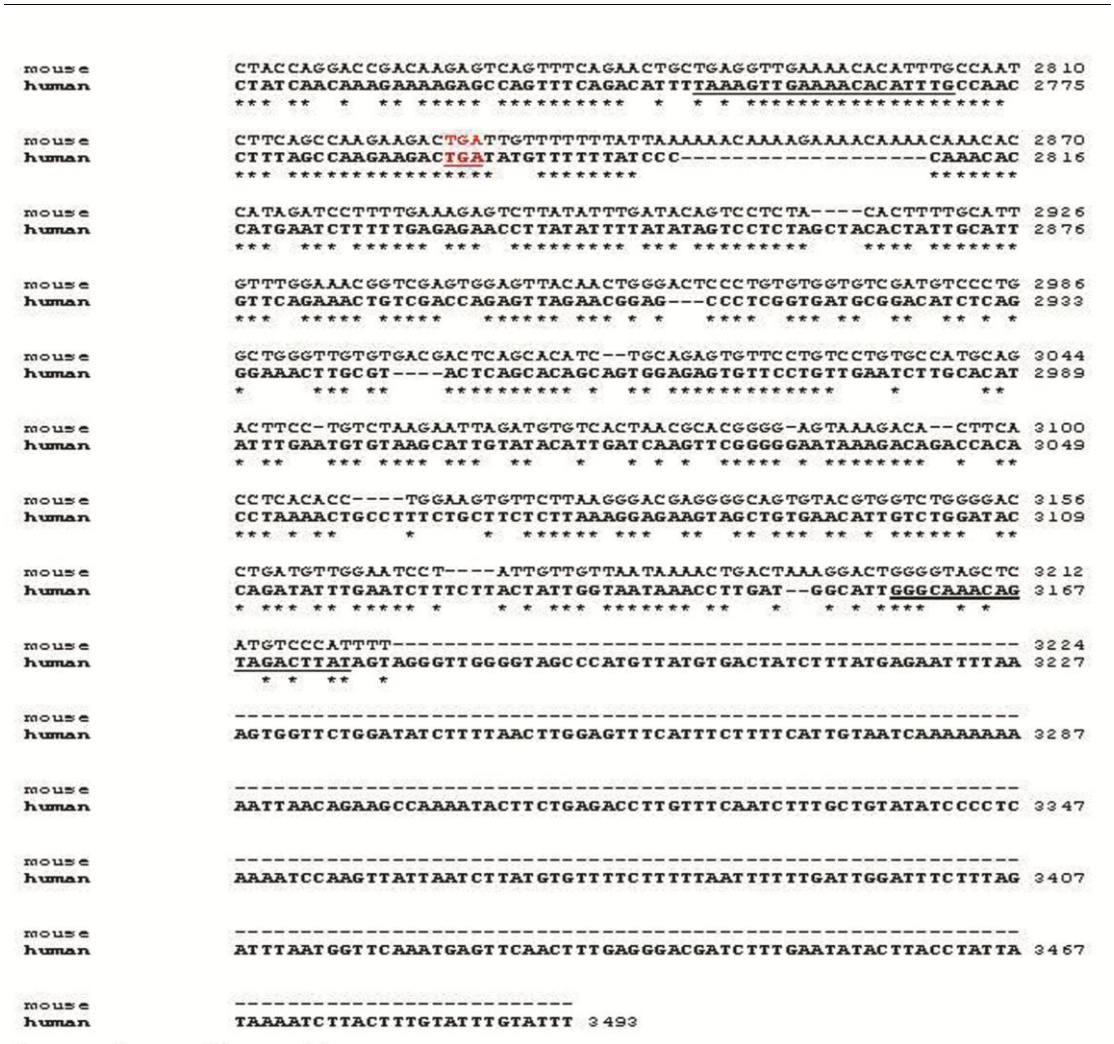
RNA probes

Nucleotides (nt) 2750 to nt 3176 of the *ENPP1* cDNA was amplified from plasmid prk7-*ENPP1* (kindly provided by Dr. Ira Goldfine, San Francisco, CA), cloned into pCRII-TOPO vector (Invitrogen) and then excised with EcoRI, and subcloned into pGEM-3Zf(+) vector (Promega) downstream of a T7 RNA polymerase promoter. M13 forward and M13 reverse primers were used to obtain a fragment including the *ENPP1* and the PCR product used for preparation of ^{32}P-labeled RNA probe by using Maxiscript T7 Kit (Ambion). The RNA probe was purified by 8M urea polyacrilamide gel electrophoresis (PAGE).

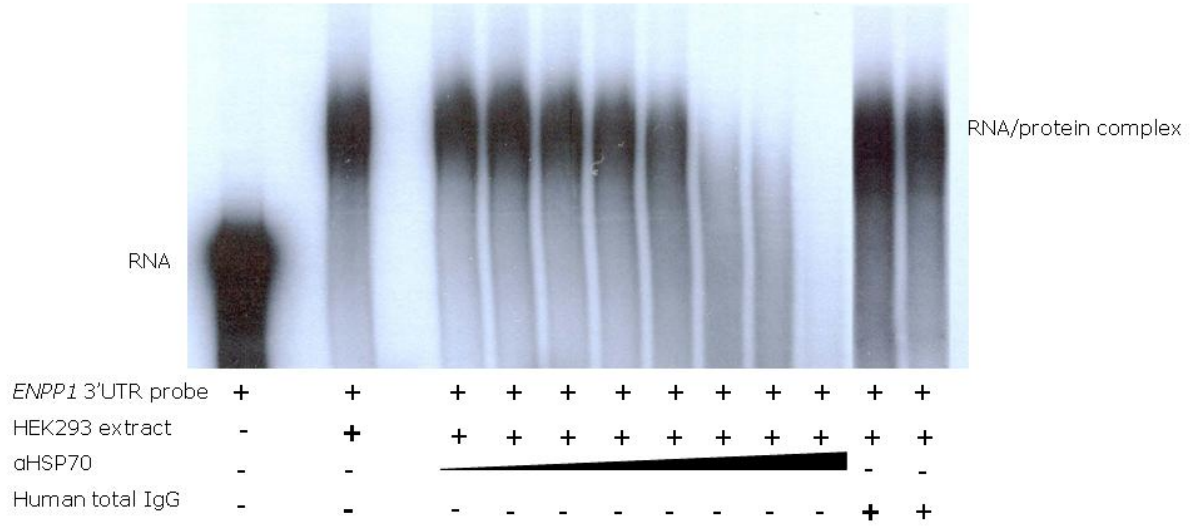
Cell culture and cell extract

Human embryo kidney 293 (HEK293) cells and human hepatocarcinoma HepG2 cells were grown in DMEM/F12 medium containing 10% FBS at 37°C with 5% CO_2. Cells were grown for 48 hours in 75-cm^2 culture flasks, seeded into six well/plates and cell extracts obtained as previously described by Mondino A, Jenkins MK (1995) *J Biol Chem* 270: 26593-26601.

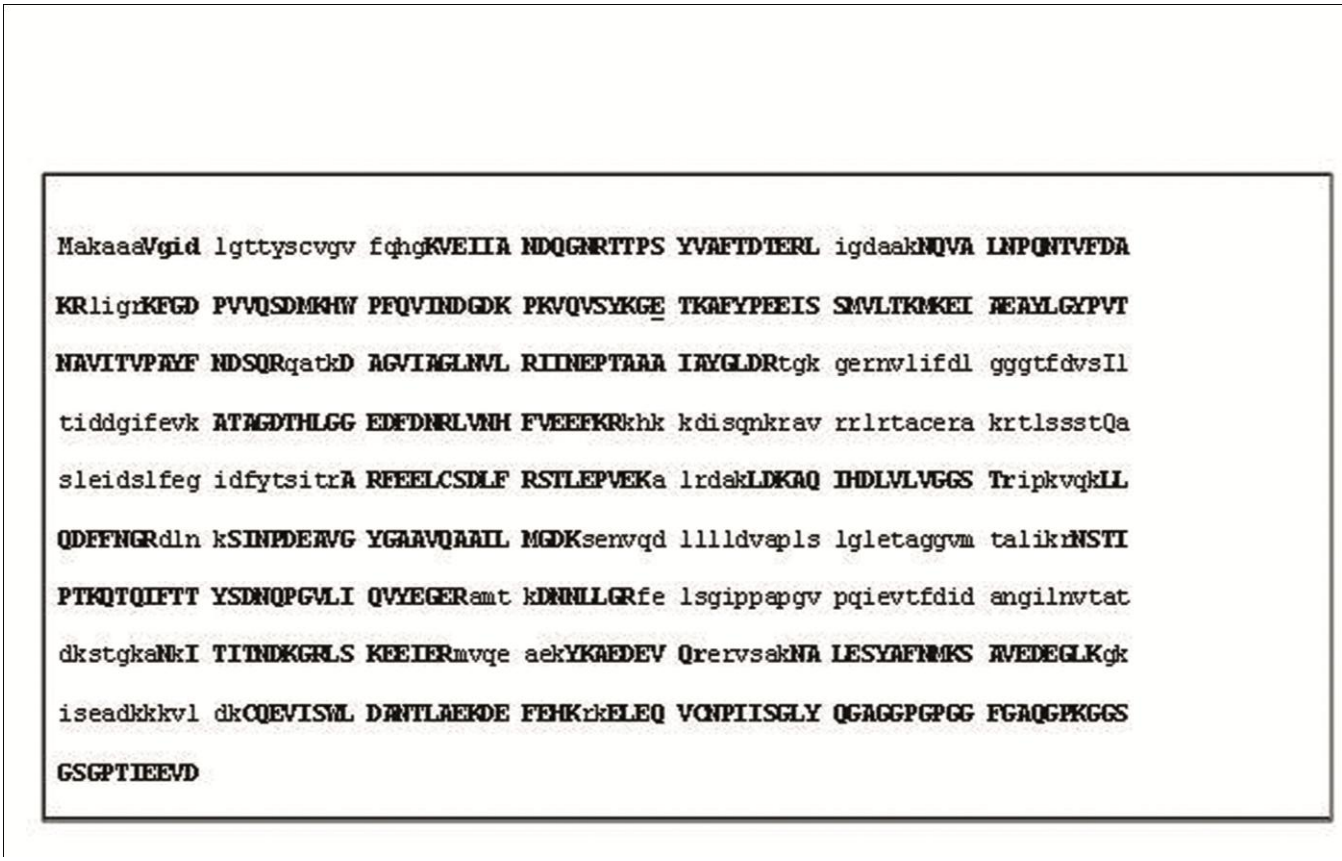
SUPPLEMENTARY FIGURES



Supplementary figure 1. Sequence comparison of mouse *Enpp1* and human *ENPP1* 3'UTR. Nucleotide sequences of human *ENPP1* (M57736) and mouse *Enpp1* (J02700) from position 2750 to 3493 are shown. Nucleotides identical for both mouse and human *ENPP1* are indicated by asterisk. Primers used to generate DNA template for transcribing RNA probe are underlined.



Supplementary figure 2 (lanes 1-2). Identification *ENPP1*-3'UTR-binding protein. REMSA was carried out using ^{32}P-labeled *ENPP1* probe in absence (lane 1) or presence (lane 2) of HEK293 cell extracts as described under "Methods." The observed band shifting indicated the formation of RNA-protein complex (lane 2). **(lanes 3-12). HSP70/*ENPP1*-3'UTR binding specificity.** ^{32}P-Radiolabeled *ENPP1* probe (see "Methods") was subjected to PAGE after incubating (lane 2-10) or not (lane 1) with HEK293 extracts. To examine protein/RNA binding specificity, increasing amount of HSP70 SPA-812 antibody (lanes 3–10) or total human IgG (lanes 11–12) were added to cell extracts/*ENPP1* probe mixture before running PAGE.



Supplementary figure 3. Amino acid sequence of HSP70. Uppercases show the peptides identified by tandem mass spectrometry.